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Staphylococcus aureus complex from animals and humans in three remote African regions

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Abstract

Staphylococcus schweitzeri has been recently considered to be a highly divergent *Staphylococcus aureus* clade and usually colonises nonhuman primates and bats in sub-Saharan Africa. Its transmissibility to humans remains unclear. We therefore investigated the transmission of *S. aureus* and *S. schweitzeri* among humans, domestic animals, and wildlife in three remote African regions. A cross-sectional study on nasal and pharyngeal colonisation in humans (n = 1288) and animals (n = 698) was performed in Côte d'Ivoire, Gabon, and Democratic Republic of Congo (DR Congo). Isolates were subjected to *spa* typing and multilocus sequence typing. Antimicrobial susceptibility and selected virulence factors were tested. *S. schweitzeri* was found in monkeys from all study sites but no transmission to humans was evident, despite frequent contact of humans with wildlife. In contrast, human-associated *S. aureus* sequence types (ST1, ST6, ST15) were detected in domestic animals and nonhuman primates, pointing toward a human-to-monkey transmission in the wild. The proportion of methicillin-resistant *S. aureus* (MRSA) among all *S. aureus* was 0% (Gabon), 1.7% (DR Congo), and 5.3% (Côte d'Ivoire). The majority of MRSA isolates belonged to the African clone ST88. In conclusion, we did not find any evidence for a transmission of *S. schweitzeri* from animals to humans. However, such a transmission might remain possible due to the close phylogenetic relation of humans and nonhuman primates. The ST88-MRSA clone was widespread in Côte d'Ivoire but not in Gabon and DR Congo.

Keywords: Africa, animal, MRSA, *Staphylococcus aureus*, *Staphylococcus schweitzeri*, transmission
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Introduction

Staphylococcus aureus is part of the normal flora of animals and humans, and can cause a multitude of infections ranging from superficial skin infections to invasive diseases. Colonisation is a

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risk factor for subsequent infection of the homologous strain, which is frequently found in the anterior nares [1,2].

Transmission of *S. aureus* and its variant methicillin-resistant *S. aureus* (MRSA) between animals and humans frequently has been reported in industrialised countries, particularly in regions with high densities of livestock. Not only the livestock-associated MRSA (LA-MRSA, CC398) but also community-associated MRSA (CA-MRSA, CC97) can be transmitted from mammals (i.e. pigs, cattle) and poultry to humans, where it is as pathogenic as other *S. aureus* lineages [3,4].

Recently, a highly divergent *S. aureus* clade was described in bats, monkeys, and great apes in sub-Sahara Africa, and is now considered to be a new species termed *Staphylococcus schweitzeri* [5–7]. *S. schweitzeri* has similar biochemical properties as *S. aureus* (i.e. catalase and coagulase positive), can be genotyped by *spa* typing and multilocus sequence typing, but differs from *S. aureus* at the whole-genome level and the peptidoglycan type [5,7]. An isolate of a divergent *S. aureus* clade that has been retrospectively confirmed as *S. schweitzeri* was found once in a human carrier in Gabon [8]. Close contacts of humans with animals might facilitate the cross-species transmission of *S. aureus* and *S. schweitzeri*, for instance, when preparing and consuming meat (e.g. monkeys). The extraction and consumption of wildlife ("bushmeat") is common in sub-Saharan Africa, particularly in remote regions [9]. However, the transmission of *S. aureus* and *S. schweitzeri* between animals and humans in remote African regions is unclear. The objective of this study was to investigate the population structure and transmission of *S. aureus* and *S. schweitzeri* between humans and animals in three remote regions in Côte d'Ivoire, Gabon, and Democratic Republic of Congo (DR Congo).

Materials and methods

Ethical clearance

Ethical clearance was obtained from the Comité d'Éthique Institutionnel, Centre de Recherches Médicales de Lambaréné, Gabon (CEI-MRU 001/2011), the Comité National d'Éthique et de la Recherche (CNER), Ministère de la Santé et de l'Hygiène Publique, République de Côte d'Ivoire (101 10/MSHP/CENR/P), and the Comité d'Éthique, Ministère de l'Enseignement Supérieur et Universitaire, République Démocratique du Congo (ESO/CE/018/11).

Written informed consent was signed or fingerprinted by each participant before enrolment. If the participant was illiterate or did not speak French, a local interpreter explained the study procedures. An independent witness additionally signed the consent form in these cases.

Study design

A population-based cross-sectional study was performed in Côte d'Ivoire, Gabon, and DR Congo to take nasal and pharyngeal swabs from humans and animals. The studied populations are characterised by a limited access to official documentation and health care and, with the exception of the Ivorian population, rare contact with urban civilisation. Subsistence farming and hunting are essential parts of their lifestyle.

In Côte d'Ivoire, participants were recruited and animals were sampled in eight villages (Daobly, Gahably, Gouliako, Keibly, Pauleoula, Ponan, Tai, Zaipobly) in close proximity to Taï National Park from 4/2012-10/2012 (Fig. 1).

In DR Congo, samples were taken from humans and animals in seven villages (Bekombo, Bungosani, Ipopé, Iyoko, Lompolé, Lui Kotale, Nganda) at the border of Salonga-Sud National Park from 07/2011 to 9/2011 (Fig. 1). In Côte d'Ivoire and DR Congo, domestic animals were alive and lived in the same villages as the human participants. Wildlife was dead and hunted for consumption as "bushmeat" on the same day when samples were taken.

In Gabon, Babongo pygmies were recruited in six villages (Village tranquille, Tsibanga, Ossimba, Ndougou, Soga, Egouba) in Waka National Park in 10/2011. Samples from animals were collected from 2010 to 2013 in the provinces Moyen Ogooué and Ngounié, where Waka National Park is located (Fig. 1).

All participants were included if they consented to enrolment. No exclusion criteria were applied. Demographic data and data on contact with animals (e.g. history of animal bites) were recorded in standardised questionnaires.

Bacterial isolates

Swabs were taken by gently rubbing a sterile cotton tip in circular moves against the nasal septum of the anterior nares and the pharyngeal mucosa. In Gabon, samples were stored in Amies transport medium (Transwabs, Medical Wire, Corsham, UK) until culture within a maximum of 2 days in the microbiology laboratory at the Albert Schweitzer Hospital, Lambaréné. Swabs from DR Congo and Côte d'Ivoire were stored in STTG medium in liquid nitrogen and shipped to Germany for culture. All samples were cultured on Columbia blood agar plates, Columbia CAP selective agar plates (Oxoid, Wesel, Germany), and SAID agar plates (bioMérieux, Marcy l'Etoile, France) for 18 to 36 hours at 36°C ± 1°C.

Presumptive colonies were screened for a positive catalase and agglutination test (Pastorex Staph-Plus, Bio-Rad, Marnes-la-Coquette, France). Species identification and susceptibility test (EUCAST breakpoints) were done using Vitek 2 automated systems (bioMérieux). Species of *S. aureus* was confirmed by the detection of the *nuc* gene [10]. Identification of *S. schweitzeri*

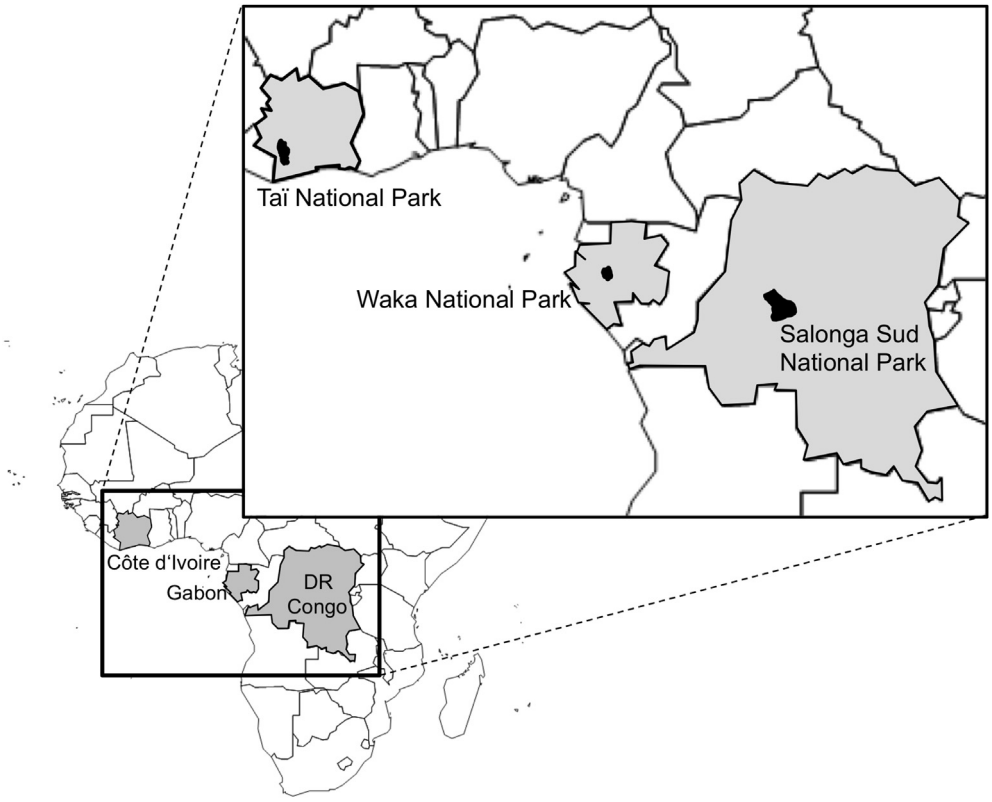

FIG. 1. Map of the study sites. The national parks in Côte d'Ivoire (Taï National Park), Gabon (Waka National Park), and Democratic Republic of Congo (Salonga Sud National Park) are shaded in black.

was based on the lack of *nuc* detection, its phylogenetic divergent position using multilocus sequence typing, and a similar biochemical profile as *S. aureus* as assessed with GP ID Card (bioMérieux) [7].

Carriers were defined as a human or animal being colonised in the nose and/or throat. If an individual was colonised with an identical isolate at different body sites (based on *spa* typing), one isolate was included in the final analysis in order to report nonduplicate isolates only.

Molecular characterisation

Genes encoding selected virulence factors were detected by multiplex polymerase chain reactions (PCRs) [11]. Methicillin resistance was confirmed by the detection of *mecA*, and all MRSA were tested for arginine catabolic mobile element [12,13]. All isolates were *spa* typed, and multilocus sequence typing (MLST) was done exemplarily for one isolate of each *spa* type in each country and each group (humans, animals) [14,15]. The concatenated sequences of MLST housekeeping genes were used to construct a Neighbour Joining Tree (MEGA5, http://www.megasoftware.net). The divergence between two groups was assessed using the Maximum Composite Likelihood model (MEGA5).

Statistics

Antimicrobial resistance rates were compared between animals and humans using Pearson's χ^2 test or Fisher exact test, the software R (http://cran.r-project.org, version: 2.13.1), and the package EPICALC.

Results

In total, 1288 participants and 698 animals were sampled in Côte d'Ivoire, Gabon, and DR Congo (Fig. 1, Table 1). The median age of humans ranged from 15 years (Gabon) to 30 years (Côte d'Ivoire). Human *S. aureus* colonisation rates were similar in Gabon (34.0%, n = 35) and Côte d'Ivoire (32.4%, n = 222) but lower in DR Congo (21.4%, n = 107). Although animal bites were not recorded for participants from DR Congo, 3.9% (n = 4, Gabon) and 20.7% (n = 142, Côte d'Ivoire) had a history of animal injuries (Table 1). The majority reported snake bites (8.2%, n = 65), followed by dog bites (4.6%, n = 36) and bites by nonhuman primates (0.9%, n = 7).

The distribution of sampled domestic animals (i.e. ruminants, fowls, dogs, cats) and wildlife (i.e. nonhuman primates, rodents, ruminants, reptiles) was heterogeneous among all study sites.

TABLE 1. Demographic data of the study populations

		Côte d'Ivoire	Gabon	Democratic Republic of Congo
Humans	Total number (n)	686	103	499
	Median age in years (range)	30 (0-80)	15 (0.5-70)	22.5 (0-87)
	Proportion of female subjects (%)	62.7	45.6	53.1
	Carrier rate (%)	32.4	34.0	21.4
	History of animal bites (%)	3.9	20.7	ND
Animal	Total number (n)	556	128	14
	Domestic animals (%)	90.1	21.4	9.4
	Wildlife (%)	9.9	78.6	90.6

ND, not done.

The proportion of domestic animals among all animals was highest in Côte d'Ivoire (90.3%, n = 501), whereas wildlife was predominant in Gabon (90.8%, n = 116) and DR Congo (88%, n = 11, Table 1). *S. aureus* colonisation rates were highest in ruminants (19.6%, n = 54), followed by nonhuman primates (19.0%, n = 15), cats (7.1%, n = 3), and dogs (3.0%, n = 3). *S. schweitzeri* was only found in nonhuman primates, which were colonised in 26.6% (n = 21).

Nine human carriers had a culture-confirmed *S. aureus* skin infection. The same strain (based on *spa* typing) as the skin lesion also was found in the nose and/or throat of six participants. Three participants with a skin infection were not colonised.

The staphylococcal isolates were separated into two phylogenetic groups. Group 1 included 495 *S. aureus* isolates predominated by ST152 (17.4%, n = 86), ST15 (16.8%, n = 83), and ST5 (10.9%, n = 54). Animal species that carried isolates belonging to group 1 were dogs (*Canis* sp.), goats (*Capra* sp.), guenons (*Cercopithecus* sp.), mangabeys (*Lophocebus* sp.), talapoins (*Miopithecus* sp.), and sheep (*Ovis* sp., Supplementary Table S1). The habitat regions of these species are terrestrial or semiterrestrial.

The divergent group 2 consisted of 24 *S. schweitzeri* isolates, and ST2295 (16.7%, n = 4), ST1872, ST1874, and ST2474 (each 8.3%, n = 2) were predominant. All *S. schweitzeri* isolates were isolated from nonhuman primates and were *nuc* PCR negative. The mean distance between group 1 and 2 was 0.083 base substitutions per site. Within group 1, we detected a cluster of monkey-associated STs (ST1838, ST1851, ST1854, ST1925, ST2721). Of these, only isolates belonging to ST1854 were *nuc* negative. Of note, two ST395 isolates from human carriers also were *nuc* negative. Isolates from wildlife and domestic animals were distinctly scattered in group 1, which is dominated by isolates from humans. In contrast, no isolates from humans were found in group 2 (Fig. 2).

S. aureus belonging to ST1, ST5, ST6, ST8, ST15, ST101, ST121, ST152, ST188, ST567, and ST1472 were found in humans, domestic animals, and/or wildlife. A possible transmission of these isolates between humans and animals was assessed by *spa* typing, which has a higher discriminatory power than MLST (Table 2). Transmission of *S. aureus* occurred more frequently between humans and domestic animals than between humans and wildlife. *S. schweitzeri* isolates were not found among humans or domestic animals (Table 2).

One early branching isolate (ST2353, *nuc* negative) from a 14-year-old female carrier in DR Congo did not cluster with any group (Fig. 2). The delineation of ST2353 from group 1, the closest related clade, was supported by a bootstrap value of 100% (Supplementary Fig. S1). The average distances of ST2353 from ST152 and ST2022 (taken as reference STs from group 1 and 2, respectively) were 0.035 and 0.054 base substitution per site, respectively. ST2353 had an isolated position regarding the MLST allelic profile; the closest relatives were ST1857, ST2022, and ST2295, which were quadruple locus variants of ST2353. ST2353 was more similar to typical *S. aureus* STs than to *S. schweitzeri*, *S. simiae*, or the early branching ST1223 and ST1850 (Fig. S1).

Antimicrobial resistance was only detected in *S. aureus* and not in *S. schweitzeri* (Table 3). Overall, the antimicrobial resistance rates were higher in *S. aureus* from humans compared with animals for penicillin (57.3 vs. 81.7%, $p < 0.005$), cefoxitin (1.1 vs. 4.7%, p 0.2) and cotrimoxazol (23.6 vs. 31.4%, p 0.15).

Compared with the pygmy population in Gabon, *S. aureus* from Côte d'Ivoire and DR Congo showed higher resistance rates to penicillin, cotrimoxazol, and tetracyclin (Table 3). The MRSA rate was 5.3% (Côte d'Ivoire) and 1.7% (DR Congo). No MRSA was detected in the Gabonese population. In Côte d'Ivoire, 94.1% (n = 16) of all MRSA belonged to ST88 (t186, t786, Panton-Valentine leukocidin (PVL) negative). One MRSA isolate was isolated from a sheep (ST2947, t186, PVL negative, Table 3). In DR Congo, the two MRSA were isolated from humans and belonged to ST8 (t1476, PVL negative, arginine catabolic mobile element (ACME) negative).

In methicillin-susceptible *S. aureus*, PVL was the most prevalent virulence factor (25% to 40.4%, Table 3). Only the enterotoxin encoding genes (*seb*, *sec*) and toxic shock syndrome toxin (*tst*) were found in *S. schweitzeri* isolates (Table 3).

Discussion

Our study assessed a possible transmission of *S. aureus* and *S. schweitzeri* among humans, domestic animals, and wildlife

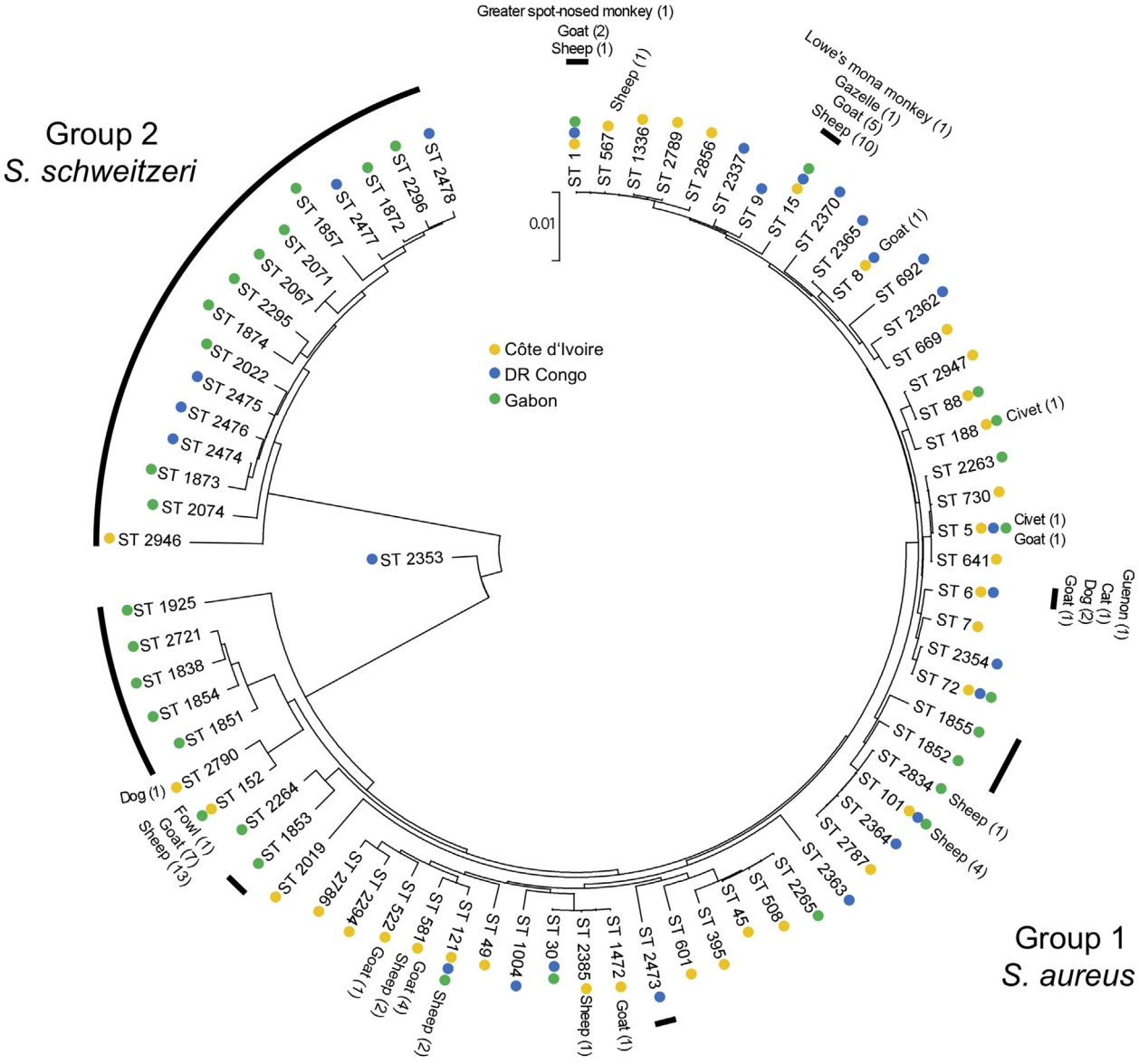

FIG. 2. Phylogenetic tree of *Staphylococcus aureus* and *Staphylococcus schweitzeri* in Côte d'Ivoire, Gabon, and DR Congo. Concatenated sequences of the seven multilocus sequence typing housekeeping genes were used to construct a neighbour-joining tree. The countries of origin are marked by colours (yellow: Côte d'Ivoire, blue: DR Congo, green: Gabon). Black lines indicate isolates from nonhuman primates.

in three remote African regions. We found evidence that transmission of *S. aureus* between humans and domestic animals could occur. *S. schweitzeri* was frequently found in nonhuman primates but was not detected in humans despite frequent exposure to bushmeat.

In human participants, 3.9% to 20.7% reported animal bites in the past, which is in line with a report from Uganda, where 19.3% reported injuries or close contact with primates [16]. Not only injuries by wild animals but also bushmeat hunting and consumption are risk factors for the transmission of pathogens between animals and humans [9]. Bushmeat trade and consumption is highest in West and Central Africa. Approximately 300 g bushmeat are consumed per person per day in the Congo Basin, resulting in 4.5 million tons of extracted bushmeat per year [9]. Therefore, our remote studied populations can be considered as populations with a high risk of becoming exposed to zoonotic pathogens from wildlife.

We and others recently reported a highly divergent *S. aureus* clade in African nonhuman primates and bats that is now considered to be a new species, *S. schweitzeri* [5,6]. *S. schweitzeri* harbours a *nuc* homologue with similar thermostable nuclease activity as *S. aureus* [17]. Despite frequent exposures to

TABLE 2. Transmission of *Staphylococcus aureus* between humans, domestic animals, and wildlife

	Human	Domestic animal		Wildlife		
MLST sequence type	***spa* type (n of isolates)**	***spa* type (n of isolates)**	**Species**	***spa* type (n of isolates)**	**Species**	**Country**
ST5	t002 (20)	—	—	t002 (1)	Civet	Democratic Republic of Congo
	t311 (6)	t311 (1)	Goat	—	—	Côte d'Ivoire
ST6	—	t1476 (5)	Cat, dog, goat	t1476 (1)	Monkey	Côte d'Ivoire
ST8	t008 (2)	t008 (1)	Goat	—	—	Côte d'Ivoire
ST15	t084 (32)	t084 (12)	Goat, sheep	t084 (1)	Monkey	Côte d'Ivoire
	t346 (8)	t346 (2)	Goat	—	—	Côte d'Ivoire
ST88/ST2947*	t186 (9)*	t186 (1)*	Sheep	—	—	Côte d'Ivoire
ST121	t314 (6)	t314 (2)	Sheep	—	—	Côte d'Ivoire
ST152	t355 (55)	t355 (16)	Chicken, dog, goat, sheep	—	—	Côte d'Ivoire
	t4235 (1)	t4235 (2)	Goat, sheep	—	—	Côte d'Ivoire
ST567	t13523 (1)	t13523 (1)	Sheep	—	—	Côte d'Ivoire
ST1472	t318 (2)	t318 (1)	Goat	—	—	Côte d'Ivoire

MLST, multilocus sequence typing.
*MRSA isolates.

nonhuman primates, we did not find *S. schweitzeri* in humans. In contrast, some isolates from monkeys clustered with typical human-associated isolates (i.e. ST1, ST6, ST15, Fig. 2). This finding suggests that a transmission from humans to animals is more likely than vice versa. This transmission could be facilitated by overlapping habitats of humans (terrestrial), domestic animals (terrestrial), and wildlife (terrestrial and semiterrestrial, Supplementary Table S1). It is also possible that animal-associated lineages were transmitted to humans, where they clonally expanded. However, the polyclonal population structure in humans argues against this scenario. As the monkeys have been hunted and handled by humans, we cannot rule out that these animals were contaminated with *S. aureus* after death and did not carry human-associated *S. aureus* lineages during lifetime. We rank this possibility as low, as swabs were taken from anterior nares and throat, which are usually not touched by humans during handling of bushmeat. However, easily accessible body sites of monkeys might remain subject to contamination by humans.

A transmission of *S. aureus* from humans to great apes has been reported from African sanctuaries and research centres in Uganda, Zambia, and Gabon [18,19]. Although direct skin contact of humans and animals might be the transmission route in captive animals, other paths might apply for a transmission to wild nonhuman primates. Contacts of wild animals with human faeces or secretions, either directly or through soil or water, could facilitate a transmission. One study from Uganda confirmed a transmission of *Escherichia coli* between humans, livestock, and gorillas [20]. In contrast, a study from Gabon did not show any evidence for such a transmission of *E. coli* from human faeces to wildlife through soil and surface waters [21].

TABLE 3. Antimicrobial resistance and virulence factors of *Staphylococcus aureus* isolates

		Côte d'Ivoire		Gabon		Democratic Republic of Congo		Total	
		***S. aureus* (n = 323)**	***S. schweitzeri* (n = 1)**	***S. aureus* (n = 52)**	***S. schweitzeri* (n = 17)**	***S. aureus* (n = 120)**	***S. schweitzeri* (n = 6)**	***S. aureus* (n = 495)**	***S. schweitzeri* (n = 24)**
Antimicrobial resistance	Penicillin	289 (89.5)	0 (0)	13 (25)	0 (0)	81 (67.5)	0 (0)	383 (77.4)	0 (0)
	Cefoxitin	17 (5.3)	0 (0)	0 (0)	0 (0)	2 (1.7)	0 (0)	20 (4.0)	0 (0)
	Cotrimoxazole	110 (34.1)	0 (0)	1 (1.9)	0 (0)	37 (30.8)	0 (0)	148 (29.9)	0 (0)
	Tetracycline	229 (70.9)	0 (0)	3 (5.8)	0 (0)	30 (25)	0 (0)	262 (52.9)	0 (0)
	Erythromycin	8 (2.5)	0 (0)	0 (0)	0 (0)	2 (1.7)	0 (0)	10 (2.0)	0 (0)
	Clindamycin	0 (0)	0 (0)	5 (9.6)	0 (0)	2 (1.7)	0 (0)	5 (1.4)	0 (0)
	Rifampicin	55 (17.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	55 (11.1)	0 (0)
Virulence factors	PVL	129 (39.9)	0 (0)	21 (40.4)	0 (0)	30 (25)	0 (0)	180 (36.4)	0 (0)
	SEA	67 (20.7)	0 (0)	2 (3.9)	0 (0)	30 (25)	0 (0)	99 (20.0)	0 (0)
	SEB	25 (7.7)	0 (0)	9 (17.3)	3 (17.6)	16 (13.3)	0 (0)	50 (10.1)	3 (12.5)
	SEC	33 (10.2)	0 (0)	6 (11.5)	2 (11.8)	12 (10)	1 (16.7)	51 (10.3)	3 (12.5)
	ETA	9 (2.8)	0 (0)	0 (0)	0 (0)	6 (5)	0 (0)	15 (3.0)	0 (0)
	ETB	2 (0.6)	0 (0)	32 (61.5)	0 (0)	3 (2.5)	0 (0)	37 (7.5)	0 (0)
	TSST	35 (10.8)	0 (0)	0 (0)	1 (5.9)	2 (1.7)	1 (16.7)	37 (7.5)	2 (8.3)
Three most frequent MLST sequence types (n)	1	ST152 (84)	ST2946 (1)	ST30 (9)	ST2295 (4)	ST5 (51)	ST2474 (2)	—	—
	2	ST15 (6)	NA	ST1 (8)	NT (2)	ST8 (16)	ST2475 (1)	—	—
	3	ST8 (26)	NA	ST1854 (4)	ST1872 (2)	ST15 (13)	ST2476 (1)	—	—

Figures are n (%) unless indicated otherwise.
Isolates that were not typable by MLST were allocated to *S. aureus* if they were *nuc* polymerase chain reaction positive [10]. Isolates being *nuc* polymerase chain reaction negative were grouped with *S. schweitzeri*.
MLST, multilocus sequence typing; NA, not applicable; NT, not typable.

In a human carrier from DR Congo, we found an early branching ST2353 that was unrelated to other early branching *S. aureus* isolates (ST1223, ST1850) [22,23]. To the best of our knowledge, similar STs have not been reported yet. The closest related STs are quadruple locus variants of ST2353 and cluster in group 2 (Fig. 2). To resolve the phylogenetic position of this ST, comparative whole-genome analyses are warranted.

The resistance rates to antimicrobial agents differed markedly between isolates from the three studied populations and between *S. aureus* and *S. schweitzeri*. There seemed to be a trend from low (Gabon), medium (DR Congo), and high (Côte d'Ivoire) levels of antimicrobial resistance in *S. aureus* that might mirror the contact with healthcare institutions and access to antibiotics. The high resistance rates to penicillin (89.5%) and cefoxitin (5.3%) in *S. aureus* from Côte d'Ivoire correspond to resistance rates in urban populations in Gabon (penicillin resistance: 95%, methicillin resistance: 3%), Nigeria (methicillin resistance: 8%), or Kenya (penicillin resistance: 69.8%, methicillin resistance: 7%) [8,24,25]. Almost all MRSA isolates from Côte d'Ivoire belonged to ST88 (94.1%). The ST88-MRSA-III/IV is widely distributed in sub-Saharan Africa and is termed the African MRSA clone [26]. We isolated an ST88-MRSA from a sheep in Côte d'Ivoire; others found this lineage in pigs from Senegal [27].

Although our study is valuable to understand the transmission of *S. aureus* and *S. schweitzeri* between animals and humans, some limitations need to be addressed. First, we were unable to include data on animal exposure and bites of the studied population in DR Congo. We assume that this population has similar exposures and contacts to wildlife as the Gabonese pygmies due to similar living conditions. Second, the cross-sectional study design limits conclusions regarding the direction of transmission. Future longitudinal studies are therefore warranted. Third, because one *spa* type can belong to different MLST sequence types, we might have underestimated the diversity of STs. Fourth, apart from the anterior nares and throat, we did not sample other typical *S. aureus* colonisation sites (e.g. hands, axilla, and groin) and might have missed additional *S. aureus* carriers.

In conclusion, we provide evidence for a transmission of human-associated *S. aureus* to domestic animals and to some extent also to wildlife in rural Africa. No transmission of the monkey-associated *S. schweitzeri* to humans was detected, although the studied populations have frequent contact with wildlife through bushmeat hunting and consumption. However, *S. schweitzeri* might have a zoonotic potential due to the close phylogenetic relation of humans and nonhuman primates.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.12.001.

References

[1] Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 2005;5:751–62.

[2] von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med 2001;344:11–6.

[3] Cuny C, Köck R, Witte W. Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. Int J Med Microbiol 2013;303:331–7.

[4] Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, et al. livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. MBio 2013;4:e00356–13.

[5] Schaumburg F, Alabi AS, Köck R, Mellmann A, Kremsner PG, Boesch C, et al. Highly divergent *Staphylococcus aureus* isolates from African non-human primates. Environ Microbiol Rep 2012;1:141–6.

[6] Akobi B, Aboderin O, Sasaki T, Shittu A. Characterization of *Staphylococcus aureus* isolates from faecal samples of the Straw-Coloured Fruit Bat (*Eidolon helvum*) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiol 2012;12:279.

[7] Tong SY, Schaumburg F, Ellington MJ, Corander J, Pichon B, Leendertz F, et al. Novel staphylococcal species that form part of a *Staphylococcus aureus* related complex: the non-pigmented *S. argenteus* and the non-human primate associated *S. schweitzeri* sp. nov. Int J Syst Evol Microbiol 2014 [in press].

[8] Ateba Ngoa U, Schaumburg F, Adegnika AA, Kösters K, Möller T, Fernandes JF, et al. Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta Trop 2012;124:42–7.

[9] Wolfe ND. Bushmeat hunting, deforestation, and prediction of zoonotic disease emergence. Emerg Infect Dis 2005;11:1822–7.

[10] Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J Clin Microbiol 1992;30:1654–60.

[11] Schaumburg F, Ngoa UA, Kösters K, Köck R, Adegnika AA, Kremsner PG, et al. Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. Clin Microbiol Infect 2011;17:1507–13.

[12] Becker K, Pagnier I, Schuhen B, Wenzelburger F, Friedrich AW, Kipp F, et al. Does nasal cocolonisation by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-positive methicillin-resistant *S. aureus* determinations by molecular methods? J Clin Microbiol 2006;44:229–31.

[13] Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant Staphylococcus aureus. Lancet 2006;367:731–9.

[14] Mellmann A, Weniger T, Berssenbrügge C, Keckevoet U, Friedrich AW, Harmsen D, et al. Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using *spa* sequence typing and the BURP (Based upon Repeat Patterns) algorithm. J Clin Microbiol 2008;46:2805–8.

[15] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008–15.

[16] Paige SB, Frost SD, Gibson MA, Jones JH, Shankar A, Switzer WM, et al. beyond bushmeat: animal contact, injury, and zoonotic disease risk in western Uganda. EcoHealth 2014 [in press].

[17] Schaumburg F, Pauly M, Schubert G, Shittu A, Tong S, Leendertz F, et al. Characterization of a novel thermostable nuclease homolog (Nuc_M) in a highly divergent *Staphylococcus aureus* clade. J Clin Microbiol 2014;52:4036–8.

[18] Schaumburg F, Mugisha L, Peck B, Becker K, Gillespie TR, Peters G, et al. Drug-resistant human *Staphylococcus aureus* in sanctuary apes pose a threat to endangered wild ape populations. Am J Primatol 2012;74:1071–5.

[19] Nagel M, Dischinger J, Türck M, Verrier D, Oedenkoven M, Ngoubangoye B, et al. Human-associated *Staphylococcus aureus* strains within great ape populations in Central Africa (Gabon). Clin Microbiol Infect 2012;19:1072–7.

[20] Rwego I, Isabirye-Basuta G, Gillespie TR, Goldberg T. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 2008;22:1600–7.

[21] Benavides JA, Godreuil S, Bodenham R, Ratiarison S, Devos C, Petretto MO, et al. No evidence for transmission of antibiotic-resistant *Escherichia coli* strains from humans to Wild Western Lowland Gorillas in Lopé National Park, Gabon. Appl Environ Microbiol 2012;78:4281–7.

[22] Holt DC, Holden MT, Tong SY, Castillo-Ramirez S, Clarke L, Quail MA, et al. A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol Evol 2011;3:881–95.

[23] Ruimy R, Armand-Lefevre L, Barbier F, Ruppé E, Cocojaru R, Mesli Y, et al. Comparisons between geographically diverse samples of carried *Staphylococcus aureus*. J Bacteriol 2009;191:5577–83.

[24] Olalekan AO, Schaumburg F, Nurjadi D, Dike AE, Ojurongbe O, Kolawole DO, et al. Clonal expansion accounts for an excess of antimicrobial resistance in *Staphylococcus aureus* colonising HIV-positive individuals in Lagos, Nigeria. Int J Antimicrob Agents 2012;40:268–72.

[25] Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JA, et al. Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. Antimicrob Resist Infect Control 2014;3:22.

[26] Schaumburg F, Alabi AS, Peters G, Becker K. New epidemiology of *Staphylococcus aureus* infection from Africa. Clin Microbiol Infect 2014;20:589–96.

[27] Fall C, Seck A, Richard V, Ndour M, Sembene M, Laurent F, et al. Epidemiology of *Staphylococcus aureus* in pigs and farmers in the largest farm in Dakar, Senegal. Foodborne Pathog Dis 2012;9:962–5.